0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(8):3232–3235

doi: 10.1210/jc.2006-0328

BRIEF REPORT

Effect of Long-Term Calorie Restriction with Adequate Protein and Micronutrients on Thyroid Hormones

Luigi Fontana, Samuel Klein, John O. Holloszy, and Bhartur N. Premachandra

Division of Geriatrics and Nutritional Sciences and Center for Human Nutrition (L.F., S.K., J.O.H.), Washington University School of Medicine, St. Louis, Missouri 63110; Division of Food Science, Human Nutrition and Health (L.F.,), Istituto Superiore di Sanitá, 00161 Rome, Italy; and Thyroid Specialty Laboratory (B.N.P.), St. Louis, Missouri 63125

Context: Caloric restriction (CR) retards aging in mammals. It has been hypothesized that a reduction in T_3 hormone may increase life span by conserving energy and reducing free-radical production.

Objective: The objective of the study was to assess the relationship between long-term CR with adequate protein and micronutrient intake on thyroid function in healthy lean weight-stable adult men and women.

Design, Setting, and Participants: In this study, serum thyroid hormones were evaluated in 28 men and women (mean age, 52 ± 12 yr) consuming a CR diet for 3–15 yr (6 ± 3 yr), 28 age- and sex-matched sedentary (WD), and 28 body fat-matched exercising (EX) subjects who were eating Western diets.

Main Outcome Measures: Serum total and free T_4, total and free T_3, reverse T_3, and TSH concentrations were the main outcome measures.

Results: Energy intake was lower in the CR group (1779 ± 355 kcal/d) than the WD (2433 ± 502 kcal/d) and EX (2811 ± 711 kcal/d) groups ($P < 0.001$). Serum T_3 concentration was lower in the CR group than the WD and EX groups (73.6 ± 22 *vs.* 91.0 ± 13 *vs.* 94.3 ± 17 ng/dl, respectively) ($P \leq 0.001$), whereas serum total and free T_4, reverse T_3, and TSH concentrations were similar among groups.

Conclusions: Long-term CR with adequate protein and micronutrient intake in lean and weight-stable healthy humans is associated with a sustained reduction in serum T_3 concentration, similar to that found in CR rodents and monkeys. This effect is likely due to CR itself, rather than to a decrease in body fat mass, and could be involved in slowing the rate of aging. **(*J Clin Endocrinol Metab* 91: 3232–3235, 2006)**

CALORIC RESTRICTION (CR) slows aging in rodents, fish, worms, and insects (1). In addition, CR has beneficial health effects in both primates and humans (2–5). Although the precise mechanisms responsible for the relationship between CR and aging processes are not known, it has been hypothesized that CR-mediated changes in endocrine function and a decrease metabolic rate are important factors (1, 2).

Thyroid hormones influence cell respiration, free radical production, and energy homeostasis (6). Although T_4 is the main product secreted by the thyroid gland, most thyroid actions are mediated by T_3. Data from studies conducted in long-lived rodents have shown that CR decreases serum T_3 concentrations, whereas serum T_4 and TSH concentrations usually remain unchanged (7, 8).

The purpose of the present study was to evaluate the thyroid hormonal profile in healthy lean and weight-stable volunteers who were consuming CR diets, containing adequate protein and micronutrients, for years. Plasma concentrations of thyroid hormones in subjects consuming a CR diet were compared with values obtained in two comparison groups: 1) age- and sex-matched sedentary subjects consuming a Western diet (WD), and 2) age-, sex-, and body fat-matched endurance runners consuming a WD.

First Published Online May 23, 2006

Abbreviations: BMI, Body mass index; CR, caloric restriction; CRP, C-reactive protein; EX, exercising; FT4, free T_4; WD, Western diet.

Subjects and Methods

Study subjects

Three groups of subjects (28 participants/group) were studied. One group (CR group) had been consuming a CR diet with adequate nutrients for a 6 ± 3 yr (range 3–15 yr) and were recruited by contacting the Calorie Restriction Society. The second group [exercising (EX) group] were endurance runners who had been running an average of 48 miles/wk (range 20–90 miles/wk) for 21 ± 11 yr (range 5–35 yr), and were recruited from the St. Louis area. The EX group was matched on age, sex, and percent body fat with the CR group. The third group (WD group) were sedentary (regular exercise < 1 h/wk) subjects, recruited from the St. Louis area who were eating a WD. The WD group was matched on age and sex with the CR and EX groups. The characteristics of the study participants are shown in Table 1. None of the participants had evidence of chronic disease, smoked cigarettes, or were taking medications or nutritional supplements that could affect the outcome variables. All participants reported weight stability, defined as less than a 2-kg change in body weight in the preceding 6 months. Serum C-reactive protein (CRP) and TNFα concentrations from 24 of 28 CR subjects and 20 of 28 WD subjects were reported previously in a study that evaluated the effects of CR on diastolic function (4), and CRP values were reported for 18 of the CR and 18 of the WD subjects in a report of the effect of CR on coronary heart disease risk factors (5). The present study was approved by the Human Studies Committee of Washington University School of Medicine, and all subjects gave informed consent before their participation

TABLE 1. Characteristics of the study subjects

	CR group (n = 28)	EX group (n = 28)	WD group (n = 28)	*P* value		
				CR *vs.* EX	CR *vs.* WD	EX *vs.* WD
Age (yr)	52.0 ± 12	52.1 ± 12	52.3 ± 10	ns	ns	ns
Sex (M/F)	24/4	24/4	24/4			
Height (m)	1.73 ± 0.1	1.75 ± 0.1	1.77 ± 0.1	ns	ns	ns
Weight (kg)	58.8 ± 5.9	68.2 ± 7.6	81.9 ± 14.6	0.003	0.0001	0.0001
BMI (kg/m^2)	19.7 ± 1.7	22.2 ± 1.9	26.0 ± 3.2	0.0001	0.0001	0.0001
TSH (mIU/liter)	1.27 ± 0.7	1.68 ± 1.0	1.24 ± 0.6	ns	ns	ns
T_3 (ng/dl)	73.6 ± 22	94.3 ± 17	91.0 ± 13	0.0001	0.001	ns
T_4 (μg/dl)	5.4 ± 1.5	5.1 ± 0.7	5.7 ± 0.9	ns	ns	ns
FT4 (ng/dl)	0.96 ± 0.14	1.0 ± 0.09	0.97 ± 0.22	ns	ns	ns
hsCRP (mg/liter)	0.23 ± 0.27	0.65 ± 0.76	1.11 ± 1.17	0.001	ns	ns
TNF-α (pg/ml)	0.74 ± 0.5	1.42 ± 1.3	1.54 ± 0.9	0.030	0.009	ns

Normal ranges: TSH, 0.47–5.0 mIU/liter; T_3, 70–165 ng/dl; T_4, 4.5–12 μg/dl; and FT4, 0.71–1.85 ng/dl. Values are means ± SD. M, Male; F, female; ns, not significant; hsCRP, high-sensitivity CRP.

Body composition

Total body fat mass and fat free mass were determined by dual-energy x-ray absorptiometry (QDR 1000/w; Hologic, Waltham, MA).

Dietary assessment

Participants recorded all food and beverage intake for 7 consecutive days. Food records were analyzed by using the Nutrition Data System from the Nutrition Coordinating Center of the University of Minnesota (version 4.03_31).

Thyroid hormones and markers of inflammation

Serum T_4 concentrations were determined by using fluorescence polarization immunoassay. Serum T_3, TSH, free T_3, and free T_4 (FT4) concentrations were determined by using microparticle enzyme immunoassay (Abbott Laboratories, North Chicago, IL). Serum rT_3 concentrations were determined by using RIA (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA). Commercially prepared ELISA kits were used to measure TNFα (Quantakine High Sensitive; R&D Systems, Minneapolis, MN), and high-sensitive CRP (ALPCO, Windham, NH) concentrations. The coefficients of variation of all assays were less than 10%.

Statistical analysis

One-way ANOVA was used to compare group variables, followed by Tukey *post hoc* testing when indicated. One-way ANOVA with Games-Howell was performed for distributions in which equal variances could not be assumed. Statistical significance was set at $P < 0.05$ for all tests. All data were analyzed by using SPSS software (version 13.0; SPSS Inc., Chicago, IL). All values are expressed as means ± SD.

Results

Body composition

Mean body mass index (BMI) values were different among the three groups (Table 1). Data obtained from body weight records kept by the CR participants showed that BMI decreased by 17%, from 23.7 ± 3 to 19.6 ± 2 kg/m^2 during the period of CR. Total body fat and truncal fat were similar in the CR and EX groups and lower than in the WD group (Table 2).

Nutrient intake

The CR subjects consumed a balance of foods that supplied more than 100% of the recommended daily intake for all the essential nutrients. Foods with a high nutrient to energy ratio such as vegetables, fruits, nuts, dairy products, egg whites, wheat and soy proteins, and meat were consumed, whereas processed foods, rich in refined carbohydrates, free sugars, and partially hydrogenated oils, were avoided. Energy intake was lower in the CR group (1779 ± 355 kcal/d; range 1112–2260 kcal/d) than in either the EX (2811 ± 711 kcal/d; range 1935–4459 kcal/d) or WD group (2433 ± 502 kcal/d; range 1756–3537 kcal/d) ($P = 0.0001$ for CR *vs.* EX or WD; $P = 0.043$ for EX *vs.* WD). Therefore, energy intake in the CR group was 27 and 37% lower than in the WD and EX groups, respectively. The percentage of total energy intake derived from protein, carbohydrate, and fat was approximately 23, 49, and 28%, respectively, in the CR group; approximately 15, 53, and 32% in the EX group; and approximately 17, 52, and 31% in the WD group. Protein intake was higher in the CR group than the WD and EX groups ($P = 0.0001$).

Thyroid hormones

Mean serum T_3 concentration was significantly lower in the CR group than the EX or WD groups, whereas serum T_4

TABLE 2. Body composition of the study subjects

		CR group (n = 28)	EX group (n = 28)	WD group (n = 28)	*P* value		
					CR *vs.* EX	CR *vs.* WD	EX *vs.* WD
Total body fat (%)	Men	6.9 ± 4.7	9.9 ± 4.7	23.7 ± 6.3		0.0001	0.0001
	Women	20.5 ± 9.9	20.0 ± 1.8	28.8 ± 7.0		ns	ns
Trunk fat (%)	Men	3.7 ± 4.4	7.0 ± 6.0	26.0 ± 8.3	ns	0.0001	0.0001
	Women	11.8 ± 8.4	13.2 ± 2.6	22.3 ± 11.0	ns	Ns	ns
Lean mass (%)	Men	92.9 ± 4.6	90.1 ± 4.7	76.3 ± 6.3	ns	0.0001	0.0001
	Women	79.4 ± 9.9	79.9 ± 1.8	71.2 ± 7.1	ns	ns	ns
Lean mass (kg)	Men	52.8 ± 4.9	59.7 ± 5.4	61.3 ± 7.5	0.001	0.0001	ns
	Women	38.9 ± 5.3	40.3 ± 3.0	39.1 ± 8.4	ns	ns	ns

Values are means ± SD. ns, Not significant.

and FT4, and TSH concentrations were not significantly different among groups (Table 1). Mean serum free T_3 concentration (normal range 1.45–3.48 pg/dl) was significantly lower in 10 CR subjects who had the lowest serum total T_3 concentrations than in 10 age- and sex-matched sedentary WD subjects (1.08 ± 0.46 *vs.* 1.68 ± 0.72 pg/ml; $P = 0.04$). However, serum rT_3 concentration (normal range 19–46 ng/dl) in 10 CR subjects who had the lowest serum total T_3 concentrations was normal and not significantly different from 10 age- and sex-matched sedentary WD subjects (26 ± 11 *vs.* 19 ± 4 ng/dl, respectively).

Markers of inflammation

Mean serum TNFα concentration was lower in the CR group than in the EX and WD groups, and mean serum CRP concentration was lower in the CR group than in the WD group (Table 1). Mean serum albumin concentration was similar in the CR (4.12 ± 0.3 g/dl), EX (4.09 ± 0.2 g/dl), and WD (4.16 ± 0.2 g/dl) groups. Mean serum prealbumin concentration in 10 CR subjects who had the lowest serum total T_3 concentrations was not significantly different from 10 age- and sex-matched sedentary WD subjects (26.9 ± 4.1 *vs.* 27.5 ± 4.5 mg/dl, respectively).

Discussion

The effect of long-term CR with adequate protein and micronutrient intake on thyroid function has not been carefully evaluated in healthy lean, weight-stable subjects. In this study, we compared the thyroid hormonal profile of men and women who were consuming a self-imposed CR diet, containing more than 100% of the recommended daily intake for all essential nutrients, for 3–15 yr, with age- and sex-matched sedentary subjects who were consuming a WD and age-, sex-, and body fat-matched endurance runners. We found that serum T_3 concentrations were lower in the CR group than sedentary or exercising subjects eating a WD. In contrast, no significant differences in serum TSH, T_4, FT4, or rT_3 were detected between groups.

Our data suggest that the mechanism responsible for the decrease in serum T_3 concentrations induced by CR is likely related to CR itself, rather than changes in body composition. Serum T_3 concentration was approximately 30% lower in the CR than the EX group, even though percent body fat was low and similar in these groups. However, energy intake was approximately 37% lower in CR than EX subjects. It has been hypothesized that energy deprivation can modulate serum T_3 concentration by reducing the activity or concentrations of iodothyronine deiodinases, which convert T_4 to T_3 (6).

Data from a series of studies have shown that short-term (2 wk to 6 months) fasting or severe CR decreases serum T_3 and transiently increases serum rT_3 concentrations in obese subjects who are actively losing weight (9). Similar findings have been reported in a study of eight nonobese individuals who unintentionally underwent moderate CR and intense physical labor (70–80 h/wk) for 21 months (3). In addition, the results from some studies (9–11) suggest that a low-carbohydrate intake (50–120 g/d) can prevent the fall in serum T_3 and particularly the rise in serum rT_3 concentration induced by CR. Carbohydrate intake in our CR subjects was approximately 250 g/d, which may have contributed to their normal serum rT_3 concentrations. Therefore, our findings provide evidence that long-term CR in sedentary lean, weight-stable subjects causes similar but persistent changes in thyroid hormones as previously reported during short-term fasting or CR in obese subjects who were continuing to experience active diet-induced weight loss.

Patients who have the sick euthyroid syndrome also have low serum T_3 concentrations (12). However, these patients have systemic nonthyroidal illnesses, such as cancer, myocardial infarction, severe infections, and major injuries (6, 12). Therefore, it is likely that inflammation, rather than decreased calorie intake, is responsible for the reduction in serum T_3 concentrations in patients with sick euthyroid syndrome (13). In fact, infusion of proinflammatory cytokines in human subjects decreases serum T_3 concentration (14, 15). Moreover, the decline in serum T_3 concentration induced by illness is blunted in IL-6 knockout mice, which supports the notion that cytokines are involved in the pathogenesis of the sick euthyroid syndrome (16). The mechanism responsible for this response is probably related to a cytokine-induced reduction in type I iodothyronine-5′-monodeiodinase expression, which results in decreased conversion of T_4 to T_3 in extrathyroidal tissues and decreased serum T_3 concentrations (6, 13–16). In contrast, low serum T_3 concentration was not associated with an increase in inflammatory cytokines in our CR subjects. In fact, markers of systemic inflammation, serum CRP and TNFα concentrations, were low in our CR subjects. These findings are consistent with data from CR studies conducted in rodents and monkeys, which showed that CR caused a marked decrease in markers of inflammation and a reduction in serum T_3 concentration (7, 8, 17, 18). The combination of decreased serum T_3 and reduced systemic inflammation could alter the aging process by reducing metabolic rate, oxidative stress, and systemic inflammation (1, 19, 20).

In conclusion, the results of this study demonstrate that long-term CR, with adequate intake of protein and micronutrients, in healthy lean and weight-stable subjects is associated with sustained low serum T_3 concentration, similar to that found in calorie-restricted rodents and monkeys. This effect is likely due to CR itself, rather than a decrease in body fat mass, and could be involved in slowing the rate of aging.

Acknowledgments

Received February 13, 2006. Accepted May 15, 2006.

Address all correspondence and requests for reprints to: Luigi Fontana, M.D., Ph.D., Washington University School of Medicine, 4566 Scott Avenue, Campus Box 8113, St. Louis, Missouri 63110. E-mail: lfontana@im.wustl.edu.

L.F. participated in the concept, design, and implementation of the study, undertook plausibility testing, and drafted the report. S.K. participated in the design and drafting of the report. J.O.H. participated in the concept, design, and implementation of the study and drafting of the report. B.N.P. participated in the design and implementation of the study and drafting of the report. All the authors declared that they participated in the study as mentioned above and that they reviewed and approved the manuscript in its final version.

This research was supported by General Clinical Research Center Grant MO1 RR00036, Diabetes Research and Training Center Grant DK20579, Clinical Nutrition Research Unit Grant DK56351, and National Institutes of Health Grant RO1 DK 37948.

The authors declare that they have no conflict of interest in connection with this paper.

References

1. **Weindruch R, Sohal RS** 1997 Caloric intake and aging. N Engl J Med 337: 986–994
2. **Mattison JA, Lane MA, Roth GS, Ingram DK** 2003 Calorie restriction in rhesus monkeys. Exp Gerontol 38:35–46
3. **Walford RL, Mock D, Verdery R, MacCallum T** 2002 Calorie restriction in biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a 2-year period. J Gerontol A Biol Sci Med Sci 57:B211–B224
4. **Meyer TE, Kovács SJ, Ehsani AA, Klein S, Holloszy JO, Fontana L** 2006 Long-term caloric restriction ameliorates the decline in diastolic function in humans. J Am Coll Cardiol 47:398–402
5. **Fontana L, Meyer TE, Klein S, Holloszy JO** 2004 Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. Proc Natl Acad Sci USA 101:6659–6663
6. **Braverman LE, Utiger RD** 2004. Werner, Ingbar's the thyroid. A fundamental and clinical text. 9th ed. New York: Lippincott
7. **Herlihy JT, Stacy C, Bertrand HA** 1990 Long-term food restriction depresses serum thyroid hormone concentrations in the rat. Mech Ageing Dev 53:9–16
8. **Maglich JM, Watson J, McMillen PJ, Goodwin B, Willson TM, Moore JT** 2004 The nuclear receptor CAR is a regulator of thyroid hormone metabolism during caloric restriction. J Biol Chem 279:19832–19838
9. **Danforth Jr E** 1986 Effects of fasting and altered nutrition on thyroid hormone metabolism in man. In: Hannemann G, ed. Thyroid hormone metabolism. New York: Marcel Dekker; 335–358
10. **Spaulding SW, Chopra IJ, Sherwin RS, Lyall SS** 1976 Effect of caloric restriction and dietary composition of serum T_3 and reverse T_3 in man. J Clin Endocrinol Metab 42:197–200
11. **Pasquali R, Parenti M, Mattioli L, Capelli M, Cavazzini G, Baraldi G, Sorrenti G, De Benedettis G, Biso P, Melchionda N** 1982 Effect of dietary carbohydrates during hypocaloric treatment of obesity on peripheral thyroid hormone metabolism. J Endocrinol Invest 5:47–52
12. **Chopra IJ** 1997 Clinical review 86: euthyroid sick syndrome: is it a misnomer? J Clin Endocrinol Metab 82:329–334
13. **Papanicolaou DA** 2000 Euthyroid sick syndrome and the role of cytokines. Rev Endocr Metab Disord 1:43–48
14. **Van der Poll T, Romijn JA, Wiersinga WM, Sauerwein HP** 1990 Tumor necrosis factor: a putative mediator of the sick euthyroid syndrome in man. J Clin Endocrinol Metab 71:1567–1572
15. **Stouthard JM, van der Poll T, Endert E, Bakker PJ, Veenhof CH, Sauerwein HP, Romijn JA** 1994 Effects of acute and chronic interleukin-6 administration on thyroid hormone metabolism in humans. J Clin Endocrinol Metab 79:1342–1346
16. **Boelen A, Maas MA, Lowik CW, Platvoet MC, Wiersinga WM** 1996 Induced illness in interleukin-6 (IL-6) knock-out mice: a causal role of IL-6 in the development of the low 3,5,3′-triiodothyronine syndrome. Endocrinology 137: 5250–5254
17. **Spaulding CC, Walford RL, Effros RB** 1997 Calorie restriction inhibits the age-related dysregulation of the cytokines TNF-α and IL-6 in C3B10RF1 mice. Mech Ageing Dev 93:87–94
18. **Roth GS, Handy AM, Mattison JA, Tilmont EM, Ingram DK, Lane MA** 2002 Effects of dietary caloric restriction and aging on thyroid hormones of rhesus monkeys. Horm Metab Res 34:378–382
19. **Tapia G, Fernandez V, Varela P, Cornejo P, Guerrero J, Videla LA** 2003 Thyroid hormone-induced oxidative stress triggers nuclear factor-κB activation and cytokine gene expression in rat liver. Free Radic Biol Med 35:257–265
20. **Chung HY, Kim HJ, Kim JW, Yu BP** 2001 The inflammation hypothesis of aging: molecular modulation by calorie restriction. Ann NY Acad Sci 928:327–335

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.